

# Behavioral dominance between female color morphs of a Lake Victoria cichlid fish

Peter D. Dijkstra,<sup>a,b</sup> Sander van Dijk,<sup>b</sup> Ton G.G. Groothuis,<sup>b</sup> Michele E.R. Pierotti,<sup>c,d</sup> and Ole Seehausen<sup>c,d</sup>

<sup>a</sup>Division of Ecology and Evolutionary Biology, Graham Kerr Building, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK, <sup>b</sup>Behavioural Biology Research Group, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands, <sup>c</sup>Institute of Ecology and Evolution, Division Aquatic Ecology and Evolution, University of Bern, Baltzerstr. 6, CH-3012 Bern, Switzerland, and <sup>d</sup>Centre of Ecology, Evolution and Biogeochemistry, Eawag Swiss Federal Institute for Aquatic Sciences, CH-6047 Kastanienbaum, Switzerland

Species that exhibit genetic color polymorphism are suitable for studying the evolutionary forces that maintain heritable phenotypic variation in nature. Male color morphs often differ in behavioral dominance, affecting the evolution of color polymorphisms. However, behavioral dominance among female color morphs has received far less attention. We studied a polymorphic population of the cichlid fish *Neochromis omnicaeruleus* from Lake Victoria, in which 3 distinct female color morphs coexist, black-and-white blotched (WB), orange blotched (OB), and plain (P) color morphs. First, we investigated dominance relationships among female morphs using triadic and dyadic encounters in the laboratory. In triadic encounters, both WB and OB females dominated plain, whereas WB females dominated OB females. Dominance of WB over OB was confirmed using dyadic encounters. In a second experiment, blotched (WB or OB) and plain full-sib sisters were bred by crossing a blotched and a plain parent. In dyadic encounters, WB female morphs dominated their plain sisters, suggesting that dominance of WB females is a pleiotropic effect of color or that genes coding for color and those influencing behavioral dominance are genetically linked, explaining the association between color and behavioral dominance despite gene flow. We conclude that behavioral dominance asymmetries exist among female color morphs of the fish *N. omnicaeruleus*, and discuss possible mechanisms that may account for the tight association between color and behavioral dominance. **Key words:** aggression, color polymorphism, dominance relationships, female–female competition, haplochromine cichlid, Lake Victoria, sexual selection. [*Behav Ecol* 20:593–600 (2009)]

Color polymorphisms within a population with 2 or more genetically based patterns or hues have been described in a variety of organisms. Examples include insects (Brockmann 2001), fish (Barlow 1983; Kingston et al. 2003), birds (Roulin 2004), and frogs (Hoffman and Blouin 2000). Such discrete polymorphisms are ideal for studying questions pertaining to the selective pressures that generate and maintain heritable phenotypic variation in nature (e.g., Hoffman and Blouin 2000; Roulin 2004; Gray and McKinnon 2007). It is often puzzling how different genetic color morphs can persist in a population, because stochastic variation or a selective advantage for 1 color should lead to the extinction of all but one (e.g., van Doorn et al. 2004). Multiple factors, such as color-based mate choice, male contest competition, and predation risk, have been invoked in explaining the maintenance of color polymorphisms through balancing and frequency-dependent selection (for review see Gray and McKinnon 2007). In many animal species, males compete for access to mates and ecological resources. Because color is often an important cue in aggressive interactions, male contest competition could have a large effect on the evolution of color (e.g., Alatalo et al. 1994; Tynkynen et al. 2004).

In several species with a discrete genetic color polymorphism, male color and dominance are correlated traits, with 1 color morph being behaviorally dominant over the other (e.g., fish: Barlow 1983; Kingston et al. 2003; Dijkstra et al. 2005, 2006; birds: Owen-Ashley and Butler 2004; Pryke and Griffith 2006; and lizards: Calsbeek and Sinervo 2002). Male coloration can then serve as a phenotypic marker, signaling social dominance of a particular color morph. The bias in the literature toward males is not surprising, because species with strong sexual selection often have colorful males but cryptically colored females. Fewer studies have investigated dominance in species where females also exhibit nonoverlapping conspicuous color morphs (Amundsen and Pärn 2006). In birds, 2 studies found evidence for asymmetric dominance relationships between female color morphs (Tuttle 2003; Pryke 2007). For instance, in the color polymorphic Gouldian finch (*Erythrura gouldiae*) red-headed females dominated both black and yellow-headed females in standardized dominance contests (Pryke 2007). However, to our knowledge, there have been no previous studies of color and behavioral dominance as correlated traits in genetic female color morphs in fish, despite the number of studies of this phenomenon in males.

One of the most intriguing, more mechanistic questions is how an association between color and dominance comes about. It may emerge when color morphs are facing different environmental conditions, such as early diet, which could affect an individual's competitive ability later in life (Royle et al. 2005). Alternatively, there could be tight genetic linkage between genes modulating aggressiveness (and others that may also affect behavioral dominance) and those coding for color. Finally, pleiotropy between color and dominance could

Address correspondence to P.D. Dijkstra, who is now at Division of Environmental and Evolutionary Biology, Graham Kerr Building, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK. E-mail: p.dijkstra@biol.gla.ac.uk.

Received 1 September 2008; revised 11 February 2009; accepted 15 February 2009.

© The Author 2009. Published by Oxford University Press on behalf of the International Society for Behavioral Ecology. All rights reserved. For permissions, please e-mail: journals.permissions@oxfordjournals.org

underlie the association. Pleiotropy, defined as phenotypic associations arising from multiple effects of the same gene(s), could be due to 2 different factors. First, certain colors, such as red, may be inherently intimidating (Barlow 1983; Hill and Barton 2005). Secondly, it is possible that hormones regulating skin pigmentation have pleiotropic effects on behavior (Sinervo and Svensson 2002; Ducrest et al. 2008). For example, it has been suggested that elevated aggression in blotched (*melanic*) mosquito fish (Horth 2003) results from  $\alpha$ -melanocyte-stimulating hormone (a melanocortin) influencing both melanin synthesis (an evolutionary conserved trait, see Takahashi and Kawachi 2006) and aggressive behavior. This is based on the observation that the  $\alpha$ -melanocyte-stimulating hormone increases sexual activity, aggression, and anxiety, at least in rats and mice (Gonzalez et al. 1996; but see Patterson et al. 1978; Tizabi et al. 1982).

Color polymorphisms are widespread in the haplochromine cichlid species flocks of the Great African Lakes. These species flocks are the most outstanding case of explosive speciation among vertebrates. Many sibling species or conspecific color morphs coexist in the same local community with marked differences in male nuptial coloration but little else (Seehausen 2000; Kocher 2004; Genner and Turner 2005). Haplochromine cichlids are female mouth brooders, and males vigorously defend individual spawning territories. Although female mate choice based on male color played a prominent role in the diversification of cichlids (e.g., Seehausen 2000), the role of male contest competition has received less attention (Seehausen and Schluter 2004; Mikami et al. 2004). However, evidence suggests it could be important in haplochromine cichlid evolution, because territory ownership and quality is important for male reproductive success (Parker and Kornfield 1996; Maan et al. 2004; Dijkstra, van der zee, and Groothuis 2008), and color is used as a cue in aggressive interactions (Dijkstra et al. 2005, 2006; Korzan and Fernald 2007; Pauers et al. 2008). Intense contest competition is also observed in female haplochromine cichlids. During the mouth brooding phase, females of many species aggressively guard their fry (Fryer and Iles 1972; Seehausen 1996). In algae-scraping species, females spend most of their time on the surface of rocks from which they scrape algae and where they can often be seen chasing one another (Ribbink 1991; Seehausen 1996; Maan et al. 2008). Suitable territories and foraging sites are likely to be scarce, resulting in interference competition among females for territories and dominance (see also, e.g., Chase et al. 2002, 2003). If females use color as a cue in their social interactions, contest competition may exert selection on female coloration.

In the rock-dwelling Lake Victoria cichlid fish *Neochromis omnicaeruleus*, several conspicuous colors are linked to dominant female determiner genes and hence are mainly found in females (Seehausen et al. 1999). In the Makobe island population (western Speke Gulf, Tanzania), 3 distinct genetic color morphs occur. They differ mainly in color but little else (Seehausen and Bouton 1997; for color plates, see Seehausen et al. 1999): a plain morph (P), and 2 conspicuously colored blotched morphs, black-and-white blotched (WB: black blotches on white), and orange blotched (OB; black blotches on orange). P is presumably the ancestral morph, because the distribution of the blotched morphs across Lake Victoria is nested within that of the P morph (Seehausen 1996). The WB and the OB morphs are predominantly found in females. Intermediates between these morphs exist but are rare. A long-term series of field data collected between 1991 and 2003 at Makobe Island suggests all 3 color morphs persist over at least 12 years (Seehausen et al. 1999; Maan et al. 2008). Fish density can be high, and females are often aggressive and territorial (Seehausen et al. 1999; Maan et al. 2008). The selective

forces that allow for the coexistence of the different color morphs remain elusive. However, in a previous study, we showed that females bias aggression toward rivals of their own color (Dijkstra, Seehausen, and Groothuis 2008). In theory, this could lead to rare females receiving fewer attacks, thereby generating negative frequency-dependent selection and stabilizing the color polymorphism (e.g., Seehausen and Schluter 2004).

In this study, we look at dominance relationships among female color morphs in the laboratory. Using triadic and dyadic encounters, we show that both types of blotched females dominate P females. We then ask how the association between color and dominance is maintained in the face of gene flow. As explained earlier, pleiotropy or tight genetic linkage between color and dominance might account for the maintenance of this association. To test this hypothesis, we stage dyadic encounters between P and blotched (WB/OB) sisters bred from a P and a blotched parent, and show that dominance is correlated with a female's color even in these full-sib contests, providing evidence for pleiotropy or genetic linkage.

## METHODS

### Species

At Makobe Island, 3 distinct color morphs occur in both sexes of *N. omnicaeruleus* (Seehausen et al. 1999). Plain females (P) are yellow to gray with 4–8 dark vertical bars (Maan et al. 2006). OB individuals have variably shaped brown blotches, superimposed on an orange or pink background. WB individuals have variably shaped black blotches on a white to brassy background. Even though about half of the females in the population are blotched, only less than 1.7% of the males are blotched. The 3 morphs are fully sympatric and ecologically indistinguishable (Seehausen and Bouton 1997; Seehausen et al. 1999; Maan et al. 2008), but differ in male and female mating preferences (Seehausen et al. 1999; Pierotti and Seehausen 2007), and mate partially assortatively in the laboratory (Seehausen et al. 1999). Males (but not females) of the WB morph exhibited mating preferences for the WB morph, and males and females of the P morph exhibited strong mating preferences for their own morph; however, males derived from a WB mother show a mating preference for WB females (Seehausen et al. 1999). Intermediate color phenotypes occur, suggesting gene flow between the morphs.

### General housing

All subjects were fully mature at the time of testing. Wild-caught females were collected at Makobe Island between May and July 2003 and brought into the laboratory for testing and breeding. They were kept in several 760-l aquaria, each housing fish of only 1 morph; lab-bred females were raised in sib-groups in 100-l aquaria. Aquarium walls, except the front, were covered with black plastic sheets so that fish in neighboring aquaria could not see each other. All aquaria were connected to a central biological filtration system through which water was circulated continuously. The water temperature was kept at  $25 \pm 2^\circ\text{C}$ , and a 12:12 h light:dark cycle was maintained. The bottom of the aquaria was covered with gravel. All fish were fed with flake food (Tetra Min Tropical Fish Flakes) daily, plus a mixture of ground shrimps and peas twice per week.

### Pre-experimentation housing

The pre-experimentation housing was as described in Dijkstra, Seehausen, and Groothuis (2008). For at least 1 week prior to the experiments, females were individually housed in separate compartments with a polyvinyl chloride (PVC) tube as a refuge. Approximately 10 compartments were made in 100 or 170 l

aquaria using transparent Perspex sheets. Females had 1 or 2 direct neighbors and visual access to all other females in the tank. The arrangement ensured that females became territorial and avoided unwanted effects of social isolation, while preventing them from engaging in physical interaction. Wild-caught females were visually exposed to females of all 3 morphs. Lab-bred females were visually exposed only to females of the color morph, which occurred in their family (e.g., a P female from a WB and P parent was only exposed to P and WB females). Test females were never housed adjacent to females with whom they would fight in subsequent tests of dominance. All females used in this study were sexually mature (i.e., older than 6 months).

### Experiment 1: triadic encounters: OB, P, and WB females

To test for behavioral dominance among the 3 female morphs, we investigated aggression in a group context by releasing trios of 1 WB, 1 OB, and 1 P morph together with 10 background fish (see below) into experimental aquaria measuring  $250 \times 66 \times 46$  cm ( $l \times w \times h$ ). The females within a trio were unrelated. The advantage of a group context in comparison with dyadic combats (see below) is that it better reflects the situation in nature, where females have brief interactions with more fish, allowing for prolonged behavioral observations (hours rather than minutes). Each trio was closely size matched (difference less than 5.5% the weight of the smallest fish). Each aquarium contained 3 vertical PVC tubes to stimulate territoriality; tubes were approximately 70 cm apart, potentially allowing 3 fish to establish a territory (Dijkstra, Hekman, et al. 2007; Dijkstra et al. 2009). We had 12 replicates, 8 of which were composed of wild-caught females and 4 of unrelated lab-bred females. Thus, we used a total of 12 OB, 12 P, and 12 WB females. Each female was used only once. We used 10 *Pundamilia* males as background fish in each experimental aquarium; for each trial, we used different background fish selected from a group of 60 *Pundamilia* males (thus, some background fish were used twice but always in a different group). These fish are standard background fish to disperse aggression in *N. omnicaruleus* in our laboratory facilities (Dijkstra, Seehausen, and Groothuis 2008).

### Observations

Females showed the same behavioral repertoire found in wrestling between male cichlids (Baerends and Baerends-Van Roon 1950; Turner and Huntingford 1986). They engaged in noncontact displays, such as lateral and frontal display and tail beating. Sometimes, escalated fighting occurred in the form of biting, mouth locking, and circling. At the end of each interaction, 1 fish would abruptly flee (defined as the loser) and was usually chased away by the other fish (defined as the winner). Observations started 15 min after the fish had been simultaneously released. We used a focal observation approach (Dijkstra, Seehausen, et al. 2007), observing each morph 5 times for a duration of 5 min, amounting to a total observation time of 25 min for each morph and replicate. The interval between each observation of an individual was at least 35 min. During observation, we recorded each aggressive interaction by noting whether the focal fish initiated a chase or was being chased (submissiveness). Submissiveness was typically associated with fleeing and a retracted dorsal fin (Baerends and Baerends-Van Roon 1950). Finally, we recorded the identity of the female(s) that defended a tube. Territory owners were defined as those who continuously patrolled around and above a tube while aggressively chasing away intruders.

### Analysis

We focused on interactions among the female color morphs only. To rank the morphs in each assemblage, we calculated

for each female her Dominance Index (defined as the number of times the individual won against an opponent divided by the total number of fights they engaged in) in relation to each of her 2 female opponents, and then took the average of these 2 values (Hemelrijk et al., 2005; Dijkstra et al. 2009). The female with the highest average Dominance Index was taken to have the highest dominance rank. To establish dominance relationships among morphs, we used a Friedman test, followed by Wilcoxon matched-pairs signed rank (WSR) tests for post hoc comparisons of ranks among the 3 female color morphs. We analyzed the rate of initiated chases and submissiveness per hour in a similar fashion. Throughout this study, all reported probabilities are for 2-tailed tests (unless stated otherwise); statistical analyses were performed in SPSS 12.0.1.

### Experiment 2: dyadic combats between OB and WB females

We staged 24 dyadic combats between unrelated OB and WB females (14 wild-caught female pairings and 10 lab-bred female pairings, using different fish in each pairing). Of these, 7 wild-caught OB and 7 wild-caught WB females were also used previously in experiment 1 with an intervening interval of at least 1 month. Although dyadic combats are somewhat more artificial than group encounters, they provide complementary information in that it allows us to investigate pairwise dominance relationships without the possible confounding factors in an assemblage situation related to the behavior of the third morph. We followed a similar procedure as described for *Pundamilia* males in Dijkstra et al. (2005). Females were quickly weighed in air on an electronic pan balance. The weight asymmetry between paired females was calculated as  $\ln(\text{weight WB}/\text{weight OB})$  following Enquist and Leimar (1983). Females were matched in pairs such that weight asymmetries were roughly symmetrically distributed over the 2 morphs (within pairs the percentage weight difference of the smallest fish ranged between 0% and 17%). Thus, although weight could differ substantially between contestants, there was no consistent weight difference between the 2 morphs (paired *t*-test,  $t = 1.182$ ,  $df = 23$ ,  $P = 0.249$ ). We staged combats by placing pairs of females in test aquaria (100 l). The females were initially separated by an opaque partition in the middle. Each compartment contained a PVC tube providing shelter. One *Pundamilia* female was placed in each compartment to maintain territoriality in the test fish; these fish were separated from the test fish by a transparent partition. All pairs were placed in the experimental aquarium 1 day before the combat took place. At the start of the combat, we removed the opaque partition between the 2 experimental fish, and also removed the PVC tubes, to avoid hiding behavior. The combat was videotaped from behind a blind. In all conflicts, we could assign a winner and a loser within 30 min. After conflicts had been settled, the fish were separated immediately. Females were weighed again to the nearest 0.1 g. These final weight measurements were used in the analysis of weight asymmetry (see above) and conflict outcome.

### Observations

All females engaged in territorial fighting when the dividing partition was removed. Females showed the same aggressive behaviors as described above. At the end of each combat, 1 fish would abruptly break from the interaction and flee; this fish was defined as the loser. The mean duration of dyadic combats between unrelated OB and WB females was  $225 \pm 33$  s (mean  $\pm$  SE (standard error),  $n = 24$ ). This was measured from the videotapes as the time period between the start of the aggressive interaction, when both fish started to display, and the time the loser first fled, minus the duration

of any pauses (Dijkstra et al. 2005). Pauses were defined as interruptions in combats during which females did not interact with each other. In such cases, they showed nonaggressive behavior, such as exploring the tank environment. To measure the sum of aggressive acts for each contestant in a dyad, we recorded from the videotapes 1) the number of covert aggressive acts, which included frontal and lateral display, and 2) the number of overt aggressive acts, which included initiation of mouth locking (the initiator being defined as the individual that was the first to lock the mouth of the other), chases (defined as a charge toward the flank of the other fish), and circling (see Baerends and Baerends-Van Roon, 1950).

### Analysis

To meet assumptions of parametric testing, we log-transformed the aggression level data, that is, sum of aggressive acts ( $\ln[x + 1]$ ). There were much larger weight differences between contestants in a dyad than in the assemblage experiment. We therefore included the weight asymmetry between the members of a dyad as a covariate in a logistic regression with contest outcome as response variable. We tested which morph had a higher probability of winning by identifying the intercept (i.e., the probability when the weight difference was zero). We compared aggression levels between female morphs, again controlling for weight asymmetry, using 2 times a repeated measure analysis of variance (RM ANOVA) (1 for overt and 1 for covert aggression), with dyads as subjects, morph as repeat (aggression of WB vs. aggression of OB), and weight asymmetry as a covariate. In none of the analyses were interaction effects significant (not reported).

### Experiment 3: dyadic combats between OB and P sisters; WB and P sisters

#### Crosses and housing

To test for pleiotropy or tight genetic linkage between color and dominance, we first generated OB female  $\times$  P male and WB female  $\times$  P male crosses. Because blotch coloration is linked to a dominant female determiner on the X chromosome (Seehausen et al. 1999) and our blotched females were heterozygous, the resulting offspring would segregate by color to produce either OB and P females (and P males) or WB and P females (and P males), respectively. This would then allow us to stage dyadic combats between OB and P full-sib sisters (from OB  $\times$  P crosses), and between WB and P full-sib sisters (from WB  $\times$  P crosses). The laboratory crossings are summarized in Table 1 in Dijkstra, Seehausen, and Groothuis (2008). We used different wild-caught fish for each cross, with 2 exceptions (see Dijkstra, Seehausen, and Groothuis, 2008). Offspring from the WB male  $\times$  P female crossing in Dijkstra, Seehausen, and Groothuis (2008) were not used in the current study. All 5 OB female  $\times$  P male crosses produced P (henceforth P<sub>ob</sub>) and OB females, and P males. All 5 WB female  $\times$  P male crosses produced P (henceforth P<sub>wb</sub>) and WB females, and P males, consistent with the existing model of inheritance (Seehausen et al. 1999). One of the WB female  $\times$  P male crosses also produced 1 WB male.

Experimental females grew up in sib-groups, guarded by their mother for the first 4 weeks posthatching in 100-l aquaria. Fish were thereafter raised in 100- or 170-l aquaria. We removed males as soon as the sexes differentiated around 6 months of age. When individuals began to mature at an age of 4–5 months, we added 10–15 juvenile *Pundamilia* sp. to each family to disperse aggression.

Table 1

Experiment 2 (WB-OB dyad) and 3 (OB-P<sub>ob</sub> and WB-P<sub>wb</sub> dyads)

Pairing	Covert aggression			Overt aggression		
	df	F	P	df	F	P
<b>WB-OB</b>						
(experiment 2)						
Weight asymmetry	1	0.002	0.961	1	0.006	0.938
Color morph	1	4.116	0.055	1	6.337	<b>0.020</b>
Error	22			22		
<b>OB-P<sub>ob</sub></b>						
(experiment 3)						
Weight asymmetry	1	0.147	0.707	1	0.044	0.837
Color morph	1	4.094	0.060	1	0.797	0.385
Error	16			16		
<b>WB-P<sub>wb</sub></b>						
(experiment 3)						
Weight asymmetry	1	1.672	0.211	1	0.981	0.334
Color morph	1	4.405	<b>0.049</b>	1	9.007	<b>0.007</b>
Error	20			21		

Results of RM ANOVAs, carried out separately for the sum of covert and overt aggressive acts in each dyad combination. The effect "color morph" represents the repeated measure indicating whether female color morphs differ consistently in the sum of aggressive acts within a pair. Significant effects are indicated in bold. OB is the orange blotched, P is the plain, and WB is the black-and-white blotched female. P<sub>ob</sub> females are P color morphs from a cross between a P and an OB parent; P<sub>wb</sub> are P color morphs from a cross between a P and a WB parent.

We then staged dyadic combats between lab-bred OB and P<sub>ob</sub> sisters ( $n = 18$  pairings), and between WB and P<sub>wb</sub> sisters ( $n = 22$  pairings), using different fish in each pairing. Within pairs of OB-P<sub>ob</sub> sisters, the percentage weight difference of the smallest fish ranged between 0% and 33%, that of pairs of WB-P<sub>wb</sub> sisters between 1% and 27%. These weight differences were substantial and therefore accounted for in the analyses (see below), although there was no consistent weight difference between any 2 morphs (paired  $t$ -test, OB-P<sub>ob</sub>:  $t = 1.401$ ,  $df = 17$ ,  $P = 0.180$ ; WB-P<sub>wb</sub>:  $t = -0.855$ ,  $df = 21$ ,  $P = 0.402$ ). The mean duration of these dyadic combats was  $205 \pm 25$  s (mean  $\pm$  SE,  $n = 40$ ).

### Observations and analysis

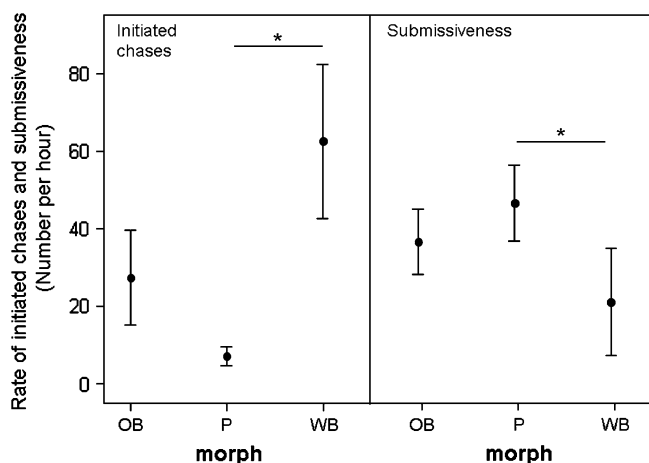
Observations and analysis were as described for the dyadic combat between OB and WB females. Aggression levels, that is, sum of aggressive acts of contestants were compared using RM ANOVAs (detailed in Results section).

## RESULTS

### Experiment 1: triadic encounters: OB, P, and WB females

All *Neochromis* female morphs behaved aggressively. When considering only the interactions among the *Neochromis* fish, the female color morphs differed in the rate of initiated chases and submissiveness (i.e., received chases), though this was only significant for the rate of submissiveness (Figure 1, Friedman test, rate of initiated chases:  $\chi^2 = 4.128$ ,  $df = 2$ ,  $P = 0.127$ ; rate of submissiveness  $\chi^2 = 9.522$ ,  $df = 2$ ,  $P = 0.009$ ,  $n = 12$ ; for pairwise comparisons of morphs see Figure 1).

The female morphs differed significantly in ranking (ranking mean  $\pm$  SE: OB female  $1.9 \pm 0.2$ ; P female  $2.6 \pm 0.5$ ; WB female  $1.5 \pm 0.2$ , Friedman test,  $\chi^2 = 9.50$ ,  $df = 2$ ,  $P = 0.009$ ,  $n = 12$ ). We found that WB significantly dominated P (WSR test,  $Z = -2.67$ ,  $P = 0.008$ ,  $n = 12$ ) and also tended to dominate OB,



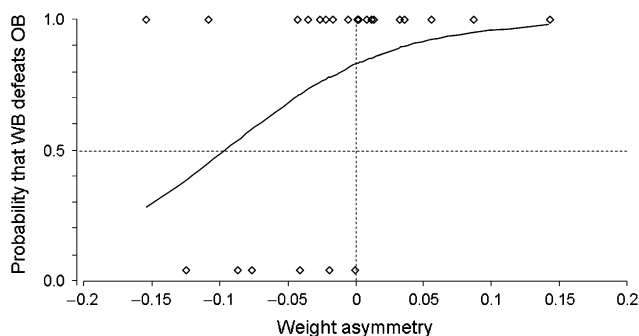
**Figure 1**

Experiment 1: rate of initiated chases (left panel) and submissiveness (right panel) for 12 female color morph trios each containing 1 OB, 1 P, and 1 WB female. Submissiveness is defined as fleeing behavior with a retracted dorsal fin as a result of being chased. Shown are the mean  $\pm$  SE. Rates are based on the interactions among the *Neochromis* female morphs only. Significant effects of pairwise comparisons are indicated with asterisks: \* $P < 0.05$ .

but this was far from significant (WSR test,  $Z = -1.100$ ,  $P = 0.272$ ). OB, in turn, significantly dominated P (WSR test,  $Z = -2.32$ ,  $P = 0.020$ ). The dominance of WB is further evident in the number of females that actively defended a tube: In total, we counted 7 WB females, 1 OB female, and 1 P female being territorial in this manner. These numbers differed significantly from random (G-test,  $G = 7.47$ ,  $P < 0.05$ ,  $df = 2$ ).

### Experiment 2: dyadic combats between OB and WB females

WB females had a probability of 0.83 of defeating OB females when weight matched; this chance was higher than expected by chance (Figure 2, intercept "Wald" = 6.29,  $df = 1$ ,  $P = 0.012$ ,  $n = 24$ ). Heavier fish tended to have a higher chance of winning, but this effect was not significant (effect weight asymmetry, Wald = 3.09,  $df = 1$ ,  $P = 0.079$ ). An RM ANOVA



**Figure 2**

Experiment 2: the probability that a female WB color morph was dominant over an OB female in dyadic combat as a function of the weight asymmetry of the 2 fish (based on the logistic regression). Weight asymmetry is expressed as  $\ln(\text{weight WB}/\text{weight OB})$ . Thus, a negative value corresponds to dyads in which OB was heavier, zero means contestants were weight matched, and a positive value indicates dyads in which WB was heavier. Values indicating winner of individual dyads (OB winner = 0, WB winner = 1) are slightly displaced for visual clarity.

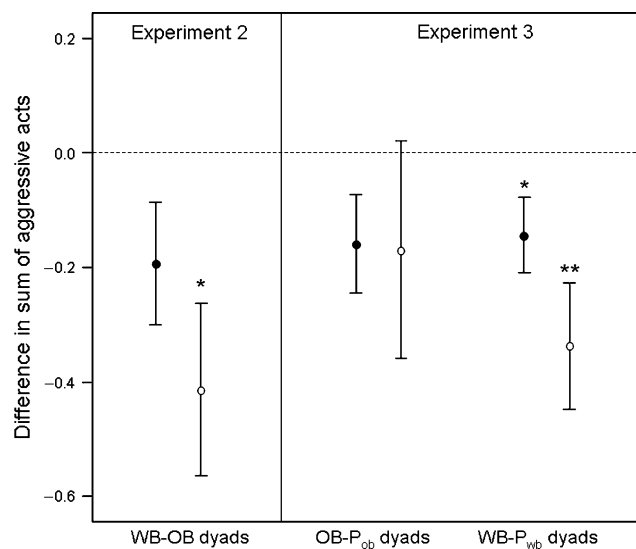
revealed that WB females were also significantly more aggressive in terms of both covert and overt aggression than OB females, whereas the weight asymmetry had no significant effect (Figure 3, for statistics see Table 1).

### Experiment 3: dyadic combats between OB and P sisters, WB and P sisters

OB females had a slightly higher probability (0.61) of winning than their  $P_{ob}$  sisters when weight matched, but this did not deviate significantly from chance (intercept, Wald = 0.897,  $df = 1$ ,  $P = 0.340$ ,  $n = 18$ ). The weight asymmetry between contestants had no influence on the outcome of the fight (Wald = 0.034,  $df = 1$ ,  $P = 0.850$ ). We ran an RM ANOVA, with the sum of aggressive acts (either covert or overt) of the OB and  $P_{ob}$  sisters as the repeated measure and weight asymmetry as the covariate. The repeated measure specifically tests whether OB females differed in aggression level from their  $P_{ob}$  sisters. We found that OB females performed more aggressive acts than their  $P_{ob}$  sisters, although not significantly so (Figure 3; for statistics see Table 1). We detected no significant effect of weight asymmetry on aggression levels (table 1).

WB females had a higher probability (0.73) of winning than their  $P_{wb}$  sisters when weight matched; this difference was higher than expected by chance (intercept, Wald = 3.903,  $df = 1$ ,  $P = 0.048$ ,  $n = 22$ ). Again, weight asymmetry between contestants had no influence on fight outcome (Wald = 0.828,  $df = 1$ ,  $P = 0.360$ ). An RM ANOVA revealed that in WB- $P_{wb}$  dyads, WB females performed significantly more aggressive acts than their  $P_{wb}$  sisters (Figure 3; for test results see Table 1). Again, we detected no significant effect of weight asymmetry on aggression levels (Table 1).

Finally, we asked whether the differences in aggression levels between WB and OB females are also expressed in their female offspring. This would be supported if combats



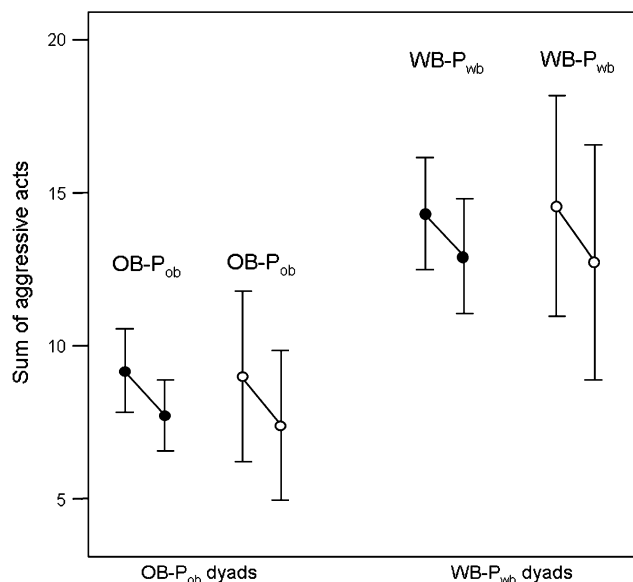
**Figure 3**

Experiments 2 and 3: difference in sum of aggressive acts between contestants. WB females were more aggressive than OB females in dyadic combats between OB and WB females; OB females were more aggressive than their plain sisters (P), but not significantly so; WB females were more aggressive than their plain sisters (P). Shown are the mean  $\pm$  SE of the difference in the sum of covert (closed circles) or overt (open circles) aggressive acts based on log-transformed data between contestants. Significant difference in the number of aggressive acts between female color morphs is indicated by an asterisk \* $P < 0.05$ , \*\* $P < 0.01$  (for statistics see Table 1).

between individuals derived from WB  $\times$  P crosses performed more aggressive acts than those between individuals derived from OB  $\times$  P crosses. In a RM ANOVA we combined the data of OB-P<sub>ob</sub> and WB-P<sub>wb</sub> fights with cross type as subjects (OB  $\times$  P-derived fish vs. WB  $\times$  P-derived fish), the sum of aggressive acts (either covert or overt) of the 2 morphs as the repeated measure and weight asymmetry as the covariate. We found that fights between WB-P<sub>wb</sub> sisters contained more aggressive acts than fights between OB-P<sub>ob</sub> sisters, although this was only significantly so for covert aggression (Figure 4, cross type, covert aggression:  $F = 4.652$ ,  $df = 1, 37$ ,  $P = 0.038$ ; overt aggression:  $F = 1.482$ ,  $df = 1, 37$ ,  $P = 0.231$ ). This interaction between the repeated measure and cross type was not significant (RM ANOVA, covert aggression:  $F = 0.072$ ,  $df = 1, 37$ ,  $P = 0.789$ ; overt aggression:  $F = 0.624$ ,  $df = 1, 37$ ,  $P = 0.435$ ), suggesting that the higher aggressiveness was expressed in both WB and their P sisters. Weight asymmetry did not affect aggression levels (RM ANOVA, weight asymmetry, covert aggression:  $F = 0.590$ ,  $df = 1, 37$ ,  $P = 0.447$ ; overt aggression:  $F = 0.879$ ,  $df = 1, 37$ ,  $P = 0.355$ ).

## DISCUSSION

In triadic encounters (experiment 1), we found 1) that WB females dominated P and showed a nonsignificant tendency to dominate OB and 2) that OB dominated P. In dyadic combats (experiment 2), WB females had a higher probability of becoming dominant than OB females. Our picture of the dominance relationships between morphs is not complete, because we lack data of OB-P and WB-P dyads using wild-caught female pairings. However, in lab-bred sister fights between females of these morphs (experiment 3), blotched females (WB/OB) tended to dominate P females, although significantly so only in WB-P fights. This can be taken as a conservative measure of dominance between blotched (WB/OB) and P females and as such is congruent with the social ranking of female color morphs in the triadic encounter experiment (WB > OB > P).



**Figure 4**  
Experiment 3: dyadic combat between WB and their P<sub>wb</sub> sisters contain more aggressive acts than those between OB and their P<sub>ob</sub> sisters. Shown are the mean  $\pm$  SE of the sum of covert (closed circles) or overt (open circles) aggressive acts for both contestants within each dyad combination.

Collectively, the data suggest that WB females are behaviorally dominant over OB and P females, and OB females are equal to or behaviorally dominant over P females.

In the triadic encounter (experiment 1), we found that the order of rank paralleled the order in the rate of chases and inversely the rate of submissiveness, with WB > OB > P. Consistently, in dyadic combat (experiment 2), the WB contestant performed more aggressive acts than the OB contestant. We found some evidence that the difference in aggression levels between OB and WB females is passed on to their offspring (experiment 3): Fights between WB and P pairings from WB  $\times$  P crosses contained far more aggressive acts than fights between OB-P pairings from OB  $\times$  P crosses, suggesting that the elevated aggressiveness of the females of the WB morph relative to the OB morph stems from a genetic difference, though we cannot rule out maternal effects as an alternative or additional explanation.

Given the evidence for considerable gene flow among the *N. omniceruleus* morphs at Makobe Island (Seehausen et al. 1999), it is puzzling how the association between color and behavioral dominance would be maintained. This could be explained when color is expressed pleiotropically with behavioral dominance. We bred individuals from either a P male and an OB female, or a P male and a WB female. The resulting offspring segregates into the 2 parental color phenotypes (Seehausen et al. 1999). In dyadic combat, WB females had a higher chance of winning than their P sisters, suggesting pleiotropy or tight genetic linkage between behavioral dominance and color in WB female morphs. OB females also tended to win more often than their P sisters in dyadic combat, but this effect was nonsignificant. It is possible that the lack of significant effects in OB-P pairings in this study is a sample size problem.

Again, the pattern in behavioral dominance was consistent with the differences in aggression levels between sister color morphs: WB performed more aggressive acts than their P sisters, whereas OB also tended to perform more aggressive acts than their P sister, although not significantly so.

How does the observed association between color and behavioral dominance come about? First, there could be tight genetic linkage between genes modulating aggressiveness (and others that may also affect behavioral dominance) and those coding for color. Secondly, there could be pleiotropy between color and dominance when certain colors have an intimidating effect on the opponent (Barlow 1983; Hill and Barton 2005). Although this effect has been mainly found for red (Cuthill et al. 1997; Pryke and Griffith 2006; Dijkstra and Preenen 2008), it is possible that the more vivid colors of the blotched female color morphs deter opponents, causing dominance (but not necessarily higher aggressiveness) to be pleiotropically expressed with color. Thirdly, it is possible that the association stems from pleiotropic effects of the genes regulating melanin synthesis on other phenotypic traits. Melanocortins underlying the dark pigmentation also influence a wide range of physiological and behavioral traits in vertebrates, including sexual behavior, aggressiveness, the stress response, immune function, and energy homeostasis (for review see Ducrest et al. 2008). A literature survey indeed showed that darker wild vertebrates are more aggressive than lighter individuals (Ducrest et al. 2008). There is some evidence that the melanocortin system plays a key role in mediating the association between color and behavioral dominance in our study species. WB is more melanized than OB (Seehausen et al. 1999), which may account for 1) behavioral dominance of WB over OB and 2) evidence for pleiotropy or genetic linkage in WB-P sister pairings, but lack of this in OB-P sister pairings. Finally, an association between color and behavioral dominance may also arise indirectly through, for example, melanocortins

enhancing immune function (Roulin et al. 2000), which may subsequently lead to improved body condition and competitive ability. These hypotheses are not mutually exclusive and are promising avenues for future studies.

Behavioral dominance of 1 phenotype over the other might facilitate the persistence or takeover of the behaviorally dominant phenotype in the population through priority in access to mates and ecological resources (Sinervo and Lively 1996; Gunckel et al., 2002; Owen-Ashley and Butler, 2004). On the other hand, female *N. omnicaruleus* of all color morphs bias their aggression toward females of the same color as their own (Dijkstra, Seehausen, and Groothuis 2008), which may stabilize the polymorphism. Preferential attack toward own-colored rivals should lead to rare phenotypes incurring fewer attacks than the more abundant female color morph, thereby generating negative frequency dependence (Seehausen and Schluter 2004; van Doorn et al. 2004; Dijkstra, Seehausen, et al. 2007). The shape and strength of this negative frequency dependence are likely affected by asymmetries in behavioral dominance between color morphs. Studies investigating the combined effect of behavioral dominance asymmetries and own-morph aggression biases on the maintenance of color polymorphisms are underway.

Other factors may account for coexistence, such as ecological factors (Galeotti et al. 2003; Galeotti and Rubolini 2004), mate choice (Tuttle 2003), or alternative reproductive tactics (Gross 1996; Sinervo and Lively 1996). For example, in the swordtail fish *Xiphophorus pygmaeus*, the gold morph dominates the blue morph, an advantage that might be counterbalanced by mating aversion of females for the gold morph (Kingston et al. 2003). In the polymorphic Gouldian finch, the red-headed morph dominated the yellow and black-headed morph, but red-headed males suffered from reduced immune function when occurring in high frequency relative to black-headed males (Pryke and Griffith 2006; Pryke 2007; Pryke et al. 2007). Together with a high degree of assortative mating by head color, dominance relationships and frequency-dependent costs may well contribute to the observed morph frequencies in the wild (Pryke 2007).

Can dominance relationships between the morphs explain the persistence of the color polymorphism in our study species? Around Makobe Island, the P color morph is in many years the most abundant female color morph (Maan et al. 2008). The persistence of the P morph suggests that any dominance advantage of blotched morphs (WB/OB) is counteracted by selection pressures favoring the P morph. Blotched females may indeed suffer several disadvantages. First, they are more conspicuous (especially OB) to visually guided predators (Maan et al. 2008). Second, blotched females produce broods with female-biased sex ratios and possibly unfit YY individuals (WB and OB are associated with dominant female determiners, see Seehausen et al. 1999).

The *N. omnicaruleus* system has inspired models of sympatric speciation by sexual selection (Lande et al. 2001; Kocher 2004, see also Seehausen et al. 1999; Pierotti and Seehausen 2007; Maan et al. 2008; Pierotti et al. 2008). If female contest competition can stabilize the persistence of the color polymorphism, it may facilitate sympatric speciation, because it would allow time for the emergence of other differences and eventually reproductive isolation between morphs (van Doorn et al. 2004; Gray and McKinnon 2007).

In summary, our study shows that behavioral dominance asymmetries exist among female color morphs of the Lake Victoria cichlid *N. omnicaruleus*. Pleiotropy or genetic linkage between color and behavioral dominance can explain the maintenance of the association of those traits despite gene flow. Testing what mechanism is responsible for the tight association is an important next step.

## FUNDING

The Netherlands Organization for Scientific Research (grant 810.64.013 and Rubicon-grant 825.07.001).

We thank Martine Maan, Mhoja Kayeba, Mohamed Haluna, Machteld Verzijden, Inke van der Sluijs, Marcel Häsler, Kees Hofker, and John Mrosso for help with the collection of live fish and discussion; Iris Bakker and Anneke Procee for help with experiments. Neil Metcalfe, Martine Maan, and 3 reviewers gave useful comments on the manuscript. The research was carried out with an animal experiment license (DEC 2812) from Groningen University and complied with current laws in The Netherlands.

## REFERENCES

- Alatalo RV, Gustafsson L, Lundberg A. 1994. Male coloration and species recognition in sympatric flycatchers. *Proc R Soc Lond B Biol Sci.* 256:113–118.
- Amundsen T, Pärn H. 2006. Female coloration: review of functional and non-functional hypotheses. In: Hill GE, McGraw KJ, editors. *Bird coloration. Function and evolution.* Vol. 2. Cambridge (MA): Harvard University Press. p. 280–348.
- Baerends GP, Baerends-Van Roon JM. 1950. An introduction to the study of the ethology of cichlid fishes. *Behaviour*. (Suppl):233–366.
- Barlow GW. 1983. Do Gold Midas cichlid fish win fights because of their color, or because they lack normal coloration? A logistic solution. *Behav Ecol Sociobiol.* 13:197–204.
- Brockmann HJ. 2001. The evolution of alternative strategies and tactics. *Adv Study Behav.* 30:1–51.
- Calsbeek R, Sinervo B. 2002. The ontogeny of territoriality during maturation. *Oecologia.* 132:468–477.
- Chase ID, Tovey C, Murch P. 2003. Two's company, three's a crowd: differences in dominance relationships in isolated versus socially embedded pairs of fish. *Behaviour.* 140:1193–1217.
- Chase ID, Tovey C, Spangler-Martin D, Manfredonia M. 2002. Individual differences versus social dynamics in the formation of animal dominance hierarchies. *Proc Natl Acad Sci USA.* 99:5744–5749.
- Cuthill IC, Hunt S, Cleary C, Clark C. 1997. Colour bands, dominance, and body mass regulation in male zebra finches. *Proc R Soc Lond B.* 264:1093–1099.
- Dijkstra PD, Hekman R, Schulz RW, Groothuis TGG. 2007. Social stimulation, nuptial coloration, androgens, and immunocompetence in a sexual dimorphic cichlid fish. *Behav Ecol Sociobiol.* 61:599–609.
- Dijkstra PD, Hemelrijk CK, Seehausen O, Groothuis TGG. 2009. Colour polymorphism and intrasexual selection in assemblages of cichlid fish. *Behav Ecol.* 20:138–144.
- Dijkstra PD, Preenen PTY. 2008. No effect of blue on winning contests in Judo. *Proc R Soc Lond B Biol Sci.* 275:1157–1162.
- Dijkstra PD, Seehausen O, Gricar BLA, Maan ME, Groothuis TGG. 2006. Can male-male competition stabilize speciation? A test in Lake Victoria cichlids. *Behav Ecol Sociobiol.* 59:704–713.
- Dijkstra PD, Seehausen O, Groothuis TGG. 2005. Direct male-male competition can facilitate invasion of new colour types in Lake Victoria cichlids. *Behav Ecol Sociobiol.* 58:136–143.
- Dijkstra PD, Seehausen O, Groothuis TGG. 2008. Intrasexual competition among females and the stabilization of a conspicuous colour polymorphism in a Lake Victoria cichlid. *Proc R Soc Lond B Biol Sci.* 275:519–526.
- Dijkstra PD, Seehausen O, Pierotti MER, Groothuis TGG. 2007. Male-male competition and speciation: aggression bias towards differently coloured rivals varies between stages of speciation in a Lake Victoria cichlid species complex. *J Evol Biol.* 20:496–502.
- Dijkstra PD, van der Zee EM, Groothuis TGG. 2008. Territory quality affects female mate preference in a Lake Victoria cichlid fish. *Behav Ecol Sociobiol.* 62:747–755.
- Ducrest A-L, Keller L, Roulin A. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends Ecol Evol.* 23:502–510.
- Enquist M, Leimar O. 1983. Evolution of fighting behaviour: decision rules and assessment of relative strength. *J Theor Biol.* 102:387–410.
- Fryer G, Iles TD. 1972. *The Cichlid fishes of the Great Lakes of Africa: their biology and evolution.* London: Oliver and Boyd.



- Galeotti P, Rubolini D. 2004. The niche variation hypothesis and the evolution of colour polymorphism in birds: a comparative study of owls, nightjars and raptors. *Biol J Linn Soc.* 82:237–248.
- Galeotti P, Rubolini D, Dunn PO, Fasola M. 2003. Colour polymorphism in birds: causes and functions. *J Evol Biol.* 16:635–646.
- Genner MJ, Turner GF. 2005. The mbuna cichlids of Lake Malawi: a model for rapid speciation and adaptive radiation. *Fish Fisheries.* 6:1–34.
- Gonzalez MI, Varizi S, Wilson CA. 1996. Behavioural effects of a -MSH and MCH after central administration in the female rat. *Peptides.* 17:171–177.
- Gray SM, McKinnon JS. 2007. Colour polymorphism and implications for speciation. *Trends Ecol Evol.* 22:71–79.
- Gross MR. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol Evol.* 11:92–98.
- Gunckel SL, Hemmingsen AR, Li JL. 2002. Effect of bull trout and brook trout interactions on foraging habitat, feeding behavior, and growth. *Trans Amer Fish Soc.* 131:1119–1130.
- Hemehrik CK, Wantia J, Gygas L. 2005. The construction of dominance order: comparing performance of five methods using an individual-based model. *Behaviour.* 142:1037–1058.
- Hill RA, Barton RA. 2005. Red enhances human performance in contests. *Nature.* 435:293.
- Hoffman EA, Blouin MS. 2000. A review of colour and pattern polymorphisms in anurans. *Biol J Linn Soc.* 70:633–665.
- Horth L. 2003. Melanic body-color and aggressive mating behavior are correlated traits in male mosquitofish, (*Gambusia holbrooki*). *Proc R Soc Lond B Biol Sci.* 270:1033–1040.
- Kingston JJ, Rosenthal GG, Ryan MJ. 2003. The role of sexual selection in maintaining a colour polymorphism in the pygmy swordtail, *Xiphophorus pygmaeus*. *Anim Behav.* 65:735–743.
- Kocher TD. 2004. Adaptive evolution and explosive speciation: the cichlid fish model. *Nat Genet.* 5:289–298.
- Korzan WJ, Fernald RD. 2007. Territorial male color predicts agonistic behavior of conspecifics in a color polymorphic species. *Behav Ecol.* 18:318–323.
- Lande R, Seehausen O, van Alphen JJM. 2001. Mechanisms of rapid sympatric speciation by sex reversal and sexual selection in cichlid fish. *Genetica.* 112–113:435–443.
- Maan ME, Eshuis B, Haesler MP, Schneider MV, Van Alphen JJM, Seehausen O. 2008. Color polymorphism and predation in a Lake Victoria cichlid fish. *Copeia.* 3:621–629.
- Maan ME, Haesler MP, Seehausen O, van Alphen JJM. 2006. Heritability and heterochrony of polychromatism in a Lake Victoria cichlid fish: stepping stones for speciation? *J Exp Zool B Mol Dev Evol.* 306B:168–176.
- Maan ME, Seehausen O, Soderberg L, Johnson L, Ripmeester AP, Mrosso HDJ, Taylor MI, van Dooren TJM, van Alphen JJM. 2004. Intraspecific sexual selection on a speciation trait, male colouration, in the Lake Victoria cichlid *Pundamilia nyererei*. *Proc R Soc Lond B Biol Sci.* 271:2445–2452.
- Mikami OK, Kohda M, Kawata M. 2004. A new hypothesis for species coexistence: male-male repulsion promotes coexistence of competing species. *Pop Ecol.* 46:213–217.
- Owen-Ashley NT, Butler LK. 2004. Androgens, interspecific competition and species replacement in hybridizing warblers. *Proc R Soc Lond B Biol Sci.* 271:S498–S500.
- Parker A, Kornfield I. 1996. Polygyny in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environ Biol Fish.* 47:345–352.
- Patterson AT, Rickerby J, Simpson J, Vickers C. 1978. MSH and the pineal in the control of territorial aggression. *J Physiol.* 285:45P.
- Pauers MJ, Kapfer JM, Fendos CE, Berg CS. 2008. Aggressive biases towards similarly coloured males in Lake Malawi cichlid fishes. *Biol Lett.* 4:156–159.
- Pierotti MER, Knight ME, Immler S, Barson NJ, Turner GF, Seehausen O. 2008. Individual variation in male mating preferences for female coloration in a polymorphic cichlid fish. *Behav Ecol.* 19:483–488.
- Pierotti MER, Seehausen O. 2007. Male mating preferences predate the origin of a female trait in an incipient species complex of Lake Victoria cichlids. *J Evol Biol.* 20:240–248.
- Pryke SR. 2007. Fiery red heads: female dominance among head color morphs in the Gouldian finch. *Behav Ecol.* 18:621–627.
- Pryke SR, Astheimer LB, Buttemer WA, Griffith SC. 2007. Frequency-dependent physiological trade-offs between competing color morphs. *Biol Lett.* 3:494–497.
- Pryke SR, Griffith SC. 2006. Red dominates black: agonistic signalling among morphs in the colour polymorphic Gouldian finch. *Proc R Soc Lond B Biol Sci.* 273:949–957.
- Ribbink AJ. 1991. Distribution and ecology of the cichlids of the African Lakes. In: Keenleyside MHA, editor. *Cichlid fishes. Behaviour, ecology and evolution*. London: Chapman and Hall. p. 36–102.
- Roulin A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol Rev.* 79:815–848.
- Roulin A, Jungi TW, Pfister H, Dijkstra C. 2000. Female barn owls (*Tyto alba*) advertise good genes. *Proc R Soc Lond B Biol Sci.* 267:937–941.
- Royle NJ, Lindström J, Metcalfe NB. 2005. A poor start in life negatively affects dominance status in adulthood independent of body size in green swordtails *Xiphophorus helleri*. *Proc R Soc B.* 272:1917–1922.
- Seehausen O. 2000. Explosive speciation rates and unusual species richness in haplochromine cichlid fishes: effects of sexual selection. *Adv Ecol Res.* 31:237–274.
- Seehausen O. 1996. Lake Victoria Rock cichlids: taxonomy, ecology and distribution. *Verduyn Cichlids.*
- Seehausen O, Bouton N. 1997. Microdistribution and fluctuations in niche overlap in a rocky shore cichlid community in Lake Victoria. *Ecol Freshwater Fish.* 6:161–173.
- Seehausen O, Schluter D. 2004. Male-male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proc R Soc Lond B Biol Sci.* 271:1345–1353.
- Seehausen O, van Alphen JJM, Lande R. 1999. Colour polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. *Ecol Lett.* 2:367–378.
- Sinervo B, Lively CM. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature.* 380:240–243.
- Sinervo B, Svensson E. 2002. Correlational selection and the evolution of genomic architecture. *Heredity.* 89:329–338.
- Takahashi A, Kawauchi H. 2006. Evolution of melanocortin systems in fish. *Gen Comp Endocrinol.* 148:85–94.
- Tizabi Y, O'Donohue TL, Jacobowitz DM. 1982. Changes in alpha-melanotropin in discrete brain areas of isolated aggressive mice. *Peptides.* 3:429–431.
- Turner GF, Huntingford FA. 1986. A problem for game theory analysis: assessment and intention in male mouthbrooder contests. *Anim Behav.* 34:961–970.
- Tuttle EM. 2003. Alternative reproductive strategies in the whitethroated sparrow: behavioral and genetic evidence. *Behav Ecol.* 14:425–432.
- Tynkkynen K, Rantala MJ, Suhonen J. 2004. Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *J Evol Biol.* 17:759–767.
- van Doorn GS, Dieckmann U, Weissing FJ. 2004. Sympatric speciation by sexual selection: a critical re-evaluation. *Am Nat.* 163:709–725.